

ISPM 27

Diagnostic protocols for regulated pests

DP 29: *Bactrocera dorsalis*

Adopted 2019; published 2019

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1. Pest Information

Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Bactrocera* Macquart consists of over 460 described species that are distributed mostly in regions of Asia and Australasia and subtropical islands of the southern Pacific Ocean (Drew and Romig, 2013; Doorenweerd *et al.*, 2018). A few *Bactrocera* species are native to Africa and several pest species were introduced to that continent. Within the genus is a group of flies named the *Bactrocera dorsalis* complex (Drew and Hancock, 1994; Drew, 2004; Clark *et al.*, 2005). This complex comprises 85 described species (Vargas *et al.*, 2015) that share a very similar appearance, but the complex as a whole does not represent a monophyletic lineage and is merely a group of convenience (Leblanc *et al.*, 2015). The complex is named after one of its member species, *Bactrocera dorsalis* (Figure 1), which is a polyphagous pest of commercial fruits. Several other species in the complex are also recognized as pests, based on plant host use and pest records (White and Elson-Harris, 1992; Clarke *et al.*, 2005; Vargas *et al.*, 2015; Plant Health Australia, 2016).

The scope of the current protocol is to diagnose adult *B. dorsalis* fruit flies. Five economically important species of the *Bactrocera dorsalis* complex that are found in commercial fruits and vegetables associated with international trade and that can be confused with *B. dorsalis* during an identification are also included in the protocol. These five species are: *B. carambolae*, *B. caryeae*, *B. kandiensis*, *B. occipitalis* and *B. pyrifoliae*. Distributions of these species are mapped with their pest status and invasion history by Vargas *et al.* (2015).

A lack of characters that can be used reliably to distinguish *B. dorsalis* from two other species (i.e. *B. papayae* Drew and Hancock, 1994 and *B. invadens* Drew *et al.*, 2005) has resulted in debate regarding the valid taxonomy of the species (Clarke *et al.*, 2005; Chen and Hui, 2007; Schutze *et al.*, 2015a, b; Drew & Romig, 2016; Schutze *et al.*, 2017). These three species have been treated as members of a sibling species complex, not to be confused with the *Bactrocera dorsalis* complex (Clarke and Schutze, 2014). It is not possible to reliably distinguish among these three species because an accurate identification requires both evaluation of species distribution information and analysis of morphological characters that are not discrete for the species. Species distribution information may not be reliable when examining specimens collected outside the species' known range. Published molecular data cannot distinguish these species (Schutze *et al.*, 2015a). In a review of available evidence, Schutze *et al.* (2015a) concluded that these three species are in fact a single biological species called *Bactrocera dorsalis*. Drew and Romig (2016) disagree with that revision and reversed the synonymy; however, Schutze *et al.* (2017) published a rebuttal to Drew and Romig (2016) that supports the synonymy by Schutze *et al.* (2015a). In this protocol, the three species are collectively treated as *B. dorsalis sensu lato*.

Evidence has been reported of hybridization among some of these six *Bactrocera* species under laboratory conditions (McInnis *et al.*, 1999; Ebina and Ohto, 2006; Schutze *et al.*, 2013) and of morphological intermediates in the wild (Delomen *et al.*, 2013; Jalani *et al.* 2014). The frequency of hybrids between these species in nature has not been estimated. Although methods for detecting hybrids between *B. dorsalis* and *B. carambolae* have been reported (Ebina and Ohto, 2006) it is currently not possible to measure impacts of hybridization events over time, such as genome introgression or detection of progeny of backcrossed populations.

B. carambolae attacks a wide range of fruits from 20 plant families, particularly *Averrhoa carambola* (carambola) (CABI, n.d.). It is found in the southern peninsular area of southeast Asia through Indonesia and several islands in the Bay of Bengal (Drew and Romig, 2013). It is also present in some South American countries (CABI, n.d.).

B. caryeae is known to attack *Mangifera* spp. (mango), *Malpighia emarginata* (acerola), *Psidium* spp. (guava), *Citrus* spp. and *Pouteria* spp. (mamey sapote), and is endemic to southern India (CABI, n.d.).

B. dorsalis s.l. attacks over 270 plant species (Vargas *et al.*, 2015) in over 50 families of commercial fruits and wild fruits (CABI, n.d.). It has the largest species range of the six pests included in this protocol, and is found on some islands in the Pacific Ocean and most of continental Africa (sub-Saharan countries) in addition to its original Asian range (Drew and Hancock, 1994; Drew *et al.*, 2005; White, 2006; Drew and Romig, 2013; Schutze *et al.*, 2015a, b).

B. kandiensis attacks a wide range of fruits including *Mangifera indica* (mango), *Garcinia* spp., *Carica papaya* (papaya), *Persea americana* (avocado) and *Psidium* spp. (guava) (CABI, n.d.). It has a limited distributional range, being endemic to Sri Lanka.

B. occipitalis attacks *Mangifera* spp. (mango), *Psidium* spp. (guava), *Spondias purpurea* (red mombin), *Averrhoa carambola* (carambola), *Citrus* spp. and *Manilkara zapota* (sapodilla) (CABI, n.d.). It has a relatively narrow range in southeast Asia (Drew and Romig, 2013).

B. pyrifoliae attacks *Psidium* spp. (guava) and *Prunus persica* (peach) (Allwood *et al.*, 1999). It is known from parts of southeast Asia (Drew and Romig, 2013).

2. Taxonomic Information

Name: *Bactrocera dorsalis* complex

Synonyms: None

Taxonomic position: Insecta, Diptera, Tephritidae, Dacinae, *Bactrocera*

The species included in the *Bactrocera dorsalis* complex are in the subgenus *Bactrocera* (*Bactrocera*). According to ICZN (1999), three species are treated as synonyms under *B. dorsalis s.l.*: *B. papayae*, *B. invadens* and *B. philippinensis*. Drew and Romig (2013) placed *B. philippinensis* as a synonym of *B. papayae*. Revision by Schutze *et al.* (2015a) placed *B. invadens* and *B. papayae* as junior synonyms of *B. dorsalis*. Drew and Romig (2016) provide an argument for treating these as separate species, but Schutze *et al.* (2017) published a rebuttal of this argument. Note that *B. invadens* was placed in the *Bactrocera dorsalis* complex by Drew *et al.* (2008) but then removed from the complex by Drew and Romig (2013). Based on Schutze *et al.* (2015a), *B. invadens* is considered a sibling species, or synonym, of *Bactrocera dorsalis*. The current protocol treats these names (*B. papayae*, *B. invadens* and *B. philippinensis*) as part of *B. dorsalis s.l.*

Table 1. Common names and synonyms of six species in the *Bactrocera dorsalis* complex included in the protocol

<i>Bactrocera</i> species	Common name	Synonyms
<i>Bactrocera (Bactrocera) carambolae</i> Drew and Hancock, 1994	Carambola fruit fly	None
<i>Bactrocera (Bactrocera) caryeae</i> (Kapoor, 1971)		<i>Chaetodacus ferrugineus incisus</i> Bezzi, 1916
		<i>Dacus (Strumeta) caryeae</i> Kapoor, 1971
<i>Bactrocera (Bactrocera) dorsalis</i> s.l. (Hendel, 1912)	Oriental fruit fly	<i>Bactrocera conformis</i> Doleschall, 1858
		<i>Dacus dorsalis</i> Hendel, 1912
		<i>Chaetodacus ferrugineus</i> var. <i>okinawanus</i> Shiraki, 1933
		<i>Dacus (Bactrocera) semifemoralis</i> Tseng, Chen & Chu, 1992
		<i>Dacus (Bactrocera) yilanensis</i> Tseng, Chen & Chu, 1992
		<i>Bactrocera papayae</i> Drew and Hancock, 1994
		<i>Bactrocera philippinensis</i> Drew and Hancock, 1994
		<i>Bactrocera invadens</i> Drew et al., 2005
<i>Bactrocera (Bactrocera) kandiensis</i> Drew and Hancock, 1994		None
<i>Bactrocera (Bactrocera) occipitalis</i> (Bezzi, 1919)		<i>Chaetodacus ferrugineus</i> var. <i>occipitalis</i> Bezzi, 1919; Drew and Hancock, 1994
		<i>Dacus (Strumeta) dorsalis</i> var. <i>occipitalis</i> (Bezzi, 1919); Hardy and Adachi, 1954; Hardy, 1969
		<i>Dacus (Strumeta) occipitalis</i> (Bezzi, 1919); Hardy, 1974
<i>Bactrocera (Bactrocera) pyrifoliae</i> Drew and Hancock, 1994		None

3. Detection

Fruit flies of the genus *Bactrocera* are detected mainly by male lure trap or in fruits. Only male adult fruit flies are captured by male lure trapping, while all immature stages such as eggs (Figure 2(a)), early to final instar larvae (Figures 2(b) to (d)), puparia and pupae (Figures 2(e) and (f)) can be found during inspection of fruits.

3.1 Trapping

Guidance on trapping *Bactrocera* fruit flies is given in Appendix 1 of ISPM 26 (*Establishment of pest free areas for fruit flies (Tephritidae)*). Additional information on trapping methods is provided by Drew (1982), Drew and Romig (2010), and FAO and IAEA (2018). The *Bactrocera dorsalis* complex includes species that respond to different male lures. When the lure responsiveness information is available, it can be used as supporting information for species identification. Five of the target species in this diagnostic protocol are methyl eugenol responding species. The only exception is *B. pyrifoliae*, which has been reported to respond to an alternative lure: cue lure (Drew and Romig, 2013).

Additional information on attractants for trapping, such as synthetic food attractants and hydrolysed protein substances, are available in Appendix 1 of ISPM 26.

3.2 Inspection of fruits

Fruits with soft areas, dark stains, dark pin spots, rot, orifices or injuries that might have originated from female oviposition or larval feeding activities should be targeted for inspection. In order to detect punctures made by female flies during oviposition, fruits should be examined under a microscope by an expert. If larval exit holes are observed, the fruit containers should be inspected for pupae. Second and third instar larvae and pupae are not likely to occur when unripe fruits are collected and packed; however, these fruits might host eggs and first instar larvae, which are more difficult to detect. Potentially infested fruits that show typical punctures made by ovipositing female flies (Figure 3) should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits.

Once detected, immature larvae can be reared to adults for identification (section 3.3). Rearing of adults is required to accurately identify a fly to species level or as part of the *Bactrocera dorsalis* complex. The incubation of infested fruits is a common practice to obtain adult flies, which is necessary to identify species in this protocol. Even if there are no signs of fruit fly infestation, an incubation could be conducted as an oviposition mark is often difficult to recognize.

3.3 Rearing larvae to obtain adults

Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g. damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should be observed and pupae gathered daily. The pupae are placed in containers with the pupation medium, and the containers are covered with a tight lid that enables proper ventilation. Once the adults emerge, they must be kept alive for several days to ensure that the tegument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. Prior to mounting (before they harden), it is useful to gently squeeze the apical part of the preabdomen with forceps, then squeeze the base and apex of the oviscape to expose the aculeus tip for females, and to pull out the aedeagus for males. Alternatively, this will need to be dissected later in flies.

4. Identification

Identification at the level of the species or the *Bactrocera dorsalis* complex requires morphological examination of adult flies. It is generally difficult and not reliable to morphologically identify eggs, larvae or pupae to the species level. It is not possible to identify a fly to the *Bactrocera dorsalis* complex using immature life stages.

Molecular methods of *Bactrocera* species identification have been reported and provide additional information to support morphological identifications of specimens. DNA sequencing of the *cytochrome oxidase I* DNA barcode does not provide adequate resolution to identify many species in the *B. dorsalis* complex (details in section 4.4). Other molecular methods lack the specificity data needed to demonstrate that a test is accurate for species identification. For example, the molecular profiles of all six pest species targeted in this protocol are not known using ribosomal DNA analysis (section 4.4). DNA can be used to distinguish *B. carambolae* from *B. dorsalis* s.l. and a method for doing this is provided in this protocol (section 4.3.2). The use of a fly leg for DNA extraction is recommended when molecular data are to be collected. For guidance on preparing a specimen for molecular study, see section 4.3.1.

4.1 Preparation of adults for identification

Proper preparation of specimens is essential for accurate morphological identification. General instructions on preparation of adult fruit fly specimens are given by Drew (1991) and White and Elson-Harris (1992).

Every attempt should be made to preserve all characters on at least one side of the centre line, regardless of the mounting method (Foote *et al.*, 1993).

Characters on the head, wing, leg, thorax and abdomen of a fly can be examined from pinned specimens under magnification using a stereomicroscope at $\geq 20\times$. This magnification level is appropriate for observation of spot and colour patterns and wing morphology (Figure 1). Microscopic examination is required to measure characters on the genitalia that are described in section 4.1.1.

Structures of the ovipositor such as the oviscapae, eversible membrane and aculeus have been used as important taxonomic characters at species level (Hardy, 1949, 1969; Hardy and Adachi, 1954; Drew and Hancock, 1994). Since the review by Drew and Hancock (1994), aculeus length has been used in particular for distinguishing some of the fruit fly species within the *Bactrocera dorsalis* complex, and male aedeagus length, which is highly correlated with aculeus length, has also been used because only males are trapped in lure trapping surveys. Care must be taken when interpreting genitalic morphometric information for species diagnostics, as some members of the *B. dorsalis* complex exhibit a wide range of aedeagus lengths over their geographical distribution (Krosch *et al.*, 2013; Schutze *et al.*, 2015a). Preparation methods for male genitalia are included in section 4.1.1.

To assist in identification of characters under a stereomicroscope, the following can be applied:

- Examination of the costal band below the R_{2+3} vein will be made easier by putting white paper underneath the wing or by using transmitted light.
- When black markings on abdominal tergites 3–5 are difficult to observe due to damage such as colour change, observation may be made easier by wetting with a paintbrush dipped in 70% ethanol or clearing with 10% potassium hydroxide (KOH).
- When the inner yellow membrane in lateral vittae (Figure 4) is partially removed, which makes the boundary of the lateral vittae difficult to see, an alternative is to measure the width of the translucent window in the scutum (Figure 5(b)).
- In measuring the width of lateral vittae (example of measuring indicated in Figure 5(b)), adjustment of the angle to give the widest value of the vittae is important.

4.1.1 Preparation of adults for microscopic examination of genitalia

The procedures for dissection of the genitalia are mainly based on White and Elson-Harris (1992), White and Hancock (1997), and Foote *et al.* (1993). When measuring the length of genitalia, it is recommended that the relative length to body size also be calculated. The length of the CuA_1 vein along the discal medial cell of the wing has been used as an index of body size in prior studies (Ebina and Ohto, 2006).

Preparation of the abdomen for dissection and examination of genitalia can be accomplished by first removing the abdomen from the specimen and soaking it in a 10% solution of KOH at 95 °C for 10 to 20 minutes depending on the condition of the specimen. Once the KOH soak is complete, the digested abdomen can be transferred to a spot of glycerol.

For aculeus examination, the dissection should be carried out in a drop of glycerol with two fine forceps (or dissection needles). The oviscapae should be broken from the rest of the abdomen and then it is possible to telescope the aculeus out of the oviscapae by gently squeezing the oviscapae with one pin (Figure 6(b)). It is necessary to finish removal of the aculeus by holding the oviscapae with one pin and pulling the aculeus out with the other (for more details, see Foote *et al.*, 1993). If the telescoping method fails, the oviscapae will need to be torn open to remove the aculeus.

For aedeagus examination, it is recommended that the epandrium–surstylus assemblage (Figure 7(c)) be pulled from the rest of the abdomen. Using two pins, it is possible to straighten the aedeagus (Figure 8). It is then recommended that a small coverslip be placed over the aedeagus, leaving the epandrium, hypandrium and aedeagus base outside of the coverslip. The coverslip is carefully moved away from the epandrium so as to stretch the aedeagus out into a straight line. It is then measured from the base of the basiphallus (enclosed by the hypandrium) to just before the aedeagal glands (Figure 8(d)). In general, the aedeagus should be preserved in glycerol. However, if the specimens are to be used only for measurement, it is sufficient to glue onto a paper stage.

4.2 Morphological identification of adults

Members of the *Bactrocera dorsalis* complex are identified using a combination of morphological characters. The diagnostic characters required to complete an identification to the six species covered by this protocol and to the *Bactrocera dorsalis* complex as a whole are provided below. Additional resources on general characters for tephritid fruit fly identification are provided in White and Elson-Harris (1992).

4.2.1 Characters to identify the subgenus *Bactrocera* (*Bactrocera*)

Methods to identify fly specimens to the genus *Bactrocera* are not within the scope of the current protocol. However, proper screening of specimens is important to ensure that flies being diagnosed are within the subgenus *Bactrocera* (*Bactrocera*). The work of White and Elson-Harris (1992) provides a useful resource for those general identifications. Characters used to identify fruit flies to the tribe Dacini, including the genus *Bactrocera*, are useful in the identification of flies to the subgenus *Bactrocera* (*Bactrocera*). These flies have reduced chaetotaxies on the head, with ocellar (Figure 9(c)) and postocellar (Figure 9(c)) bristles absent (atrophied); the first flagellomere (Figure 9(a)) is at least three times as long as broad; and wing cell cup extension is very long (Figure 10, top). In addition to these characteristics, fruit flies of the genus *Bactrocera* have separate abdominal tergites (Figure 7(a)) (except for first and second tergites). In addition to the above characteristics of the genus *Bactrocera*, the subgenus *Bactrocera* also has the characteristics listed below.

The presence of diagnostic characters of other *Bactrocera* subgenera is useful in diagnosing flies, via exclusion, as not being members of the *Bactrocera dorsalis* complex. For example, flies in the subgenus *Bactrocera* (*Afrodacus*) lack anterior supra-alar bristles (Figure 11) and flies in the subgenus *Bactrocera* (*Gymnodacus*) lack pectens on tergite 3 (Figure 7(a)). The characters listed below are used for defining the subgenus *Bactrocera*. In starting identification, it is important to confirm that the fruit flies in question meet the definition. At this stage of identification, superficially similar species in other subgenera such as *Afrodacus* or *Gymnodacus* that could be intercepted during plant inspection can be excluded.

List of diagnostic characters of subgenus *Bactrocera* (*Bactrocera*):

- posterior lobe of male surstylus short (Figure 7(c))
- abdominal sternite 5 of male deeply concave on posterior margin (Figure 7(b))
- abdominal tergite 3 of male with pecten (Figure 7(a))
- postpronotal bristles absent (Figure 11)
- anterior supra-alar (a. sa.) bristles present (Figure 11)
- prescutellar acrostichal (prsc.) bristles usually present (Figure 11)
- one pair of apical scutellar (sc.) bristles present (Figure 11).

4.2.2 Characters to identify the *Bactrocera dorsalis* complex

Characters useful for the identification of adult flies following the terminology of Drew and Romig (2013) are listed in Table 2. The set of characters used to identify the *Bactrocera dorsalis* complex in this protocol follows Drew and Romig (2013) except for scutum colour. Scutum colour in Drew and Romig (2013) is black, but herein black and red–brown are included in the description of the complex.

A specimen must have characters that match the descriptions provided in Table 2 for the fly to be confidently identified as a *B. dorsalis* complex species.

Table 2. A combination of characters to diagnose the *Bactrocera dorsalis* complex

Structure	Description
Head	Face yellow with distinct facial spots present (Figures 9(a), 9(b), 12)
Scutum	Colour mostly black to mostly red–brown (inter-regionally variable) (Figure 13)
	Lateral vittae present (Figure 11) and yellowish (Figures 13 and 14)
	Medial vittae absent (Figure 11)
Scutellum	Yellowish colour (Figures 1 and 13)
	With a dark basal band (Figures 11 and 13)
	Never with other dark patterns (Figure 13)
Femora	Entirely or mostly fulvous (reddish-yellow or tawny) colour but may possess dark patterns particularly on and around apices (Figure 15)
Wing	Cells bc and c hyaline (colourless) or, at most, with an extremely pale tint (Figures 10 and 16)
	Without dense microtrichia covering cells bc and c (Figure 10)
	Costal band narrow (never confluent with R ₄₊₅) (Figure 10)
	Narrow anal streak present (diagonal marking that is above anal lobe) (Figures 10 and 16)
Abdomen	With a “T” pattern on tergites 3–5 (Figures 7(a) and 17)

4.2.3 Morphological identification of six economically important species belonging to the *Bactrocera dorsalis* complex

Morphological identification of species in the *Bactrocera dorsalis* complex is difficult in part because of a high level of character variability within species and overlap in characters between species. Ranges of variations in each diagnostic character shown in Table 3 are compiled from various sources including Drew and Hancock (1994), Drew and Romig (2013, 2016), and Schutze *et al.* (2015a, b). In Table 3, some character descriptions are recorded with indications of being “inter-regionally” or “intra-regionally” variable because some of the regional populations seem to have clearly unique variations in qualitative or quantitative characters.

Identification at species level is generally difficult when specimens lack a combination of characteristics typical for one of the species. This is particularly evident in diagnosis of *B. dorsalis s.l.* and *B. carambolae* when genitalia lengths can match either species. As mentioned, hybrids are possible between these species but cannot be diagnosed with confidence using morphology.

An identification to one of the six species in the protocol requires the adult specimen to be examined for the characters provided in Table 3. This can be accomplished using the key in section 4.2.4 to screen specimens and then identification can be confirmed by comparing fly morphology to information in Table 3. If one or more characters are inconsistent between the specimen and the descriptions provided in Table 3, then the specimen cannot be diagnosed as one of these species. Morphometric examination of genitalia does not always provide a clear diagnosis because of overlap in the range of aedeagus and aculeus sizes between *B. dorsalis s.l.* and *B. carambolae* (Table 3). These characters are included because they can be informative in distinguishing some specimens of *B. dorsalis s.l.* from *B. carambolae*. When specimens match both *B. dorsalis s.l.* and *B. carambolae* based on morphology, then a molecular test (section 4.3) should be run to distinguish between these species.

Table 3. Diagnostic morphological characters of adult fruit flies of six economically important species of the *Bactrocera dorsalis* complex

Structure	Species					
	<i>Bactrocera carambolae</i>	<i>Bactrocera caryeae</i>	<i>Bactrocera dorsalis</i> s.l.	<i>Bactrocera kandiensis</i>	<i>Bactrocera occipitalis</i>	<i>Bactrocera pyrifoliae</i>
Head						
Facial spots (Figures 9(a), 9(b), 12)	Medium-sized, oval (Figure 12(a))	Large, elongate oval (Figure 12(b))	Medium to large, circular to oval (inter-regionally variable) (Figure 12(c))	Large, oval (Figure 12(d))	Large, oval (Figure 12(e))	Medium-sized, circular (Figure 12(f))
Abdomen						
Tergites 3–5 (Figures 7(a), 17, 18)	With medium-width medial longitudinal black stripe (Figures 17(a) and 18(a))	With narrow medial longitudinal black stripe (Figures 17(b) and 18(b))	With narrow to medium-width medial longitudinal black stripe (Figures 17(c) and 18(c))	With very narrow medial longitudinal black stripe (Figures 17(d) and 18(d))	With very broad medial longitudinal black stripe (Figures 17(e) and 18(e))	With narrow to medium-width medial longitudinal black stripe (Figures 17(f) and 18(f))
Tergite 3	With a narrow transverse black band across anterior margin (constituting a “T” pattern) widening to cover lateral margins	With a broad transverse black band across anterior third to half	Exhibits variations from transverse black band across anterior margin (constituting a “T” pattern) to broad lateral bands	With a narrow transverse black band across anterior margin (constituting a “T” pattern)	With a narrow transverse black band across anterior margin widening to cover lateral margins	With a narrow to medium-width transverse black band widening to cover outer third of lateral margins
Tergite 4	With rectangular anterolateral (occasionally triangular) black markings	With broad lateral black bands	Without any markings or with anterolateral black markings (occasionally rectangular in shape)	With very narrow anterolateral black markings	Exhibits variations from anterolateral black markings to broad lateral bands	With a narrow to medium-width transverse black band widening to cover outer third of lateral margins
Tergite 5	With anterolateral black markings	With broad lateral black bands	Without any markings or with anterolateral black markings	With very narrow anterolateral black markings	With broad lateral black bands that cover lateral margins	With broad lateral black bands that cover lateral margins

(Table 3 continued on next page)

(Table 3 continued)

Structure	Species					
	<i>Bactrocera carambolae</i>	<i>Bactrocera caryeae</i>	<i>Bactrocera dorsalis</i> s.l.	<i>Bactrocera kandiensis</i>	<i>Bactrocera occipitalis</i>	<i>Bactrocera pyrifoliae</i>
Thorax						
Scutum colour (Figure 13)	Dull black (Figure 13(a))	Entirely black (Figure 13(b))	Black to red–brown (inter or intra-regionally variable) (Figure 13(c))	Black (Figure 13(d))	Black (Figure 13(e))	Entirely black (Figure 13(f))
Postpronotal lobe (Figures 5, 11, 13, 19)	Entirely yellow (Figure 19(a))	Yellow with dark anteromedial corner (Figure 19(b))	Entirely yellow (Figure 19(c))	Yellow with dark anteromedial corner (Figure 19(d))	Entirely yellow (Figure 19(e))	Entirely yellow (Figure 19(f))
Anterior margin of anepisternal stripe (Figures 5(a) and 14)	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; convex (anterior margin) (Figure 14(a))	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; straight (anterior margin) (Figure 14(b))	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; straight to convex (anterior margin) (Figure 14(c))	Slightly wider than notopleuron, equal in width to notopleuron; straight (anterior margin) (Figure 14(d))	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; convex (anterior margin) (Figure 14(e))	Equal in width to notopleuron; convex (anterior margin) (Figure 14(f))
Basal band of scutellum (Figures 11 and 13)	Narrow (Figure 13(a))	Moderately broad (Figure 13(b))	Narrow (Figure 13(c))	Narrow (Figure 13(d))	Narrow (Figure 13(e))	Narrow (Figure 13(f))
Lateral vittae (Figures 4, 5, 11)	Broad, parallel-sided, ending at or behind ia. bristles (Figure 4(a))	Very narrow; either entirely parallel-sided or narrowing posteriorly; ending at or just before ia. bristles (Figure 4(b))	Narrow to broad (inter-regionally variable), parallel-sided, ending at or just behind ia. bristles (Figure 4(c))	Narrow, parallel-sided, ending at ia. bristles (Figure 4(d))	Broad, parallel- or subparallel-sided; either ending at ia. bristles or (in some specimens) ending behind ia. bristles (Figure 4(e))	Narrow; either subparallel-sided and ending before ia. bristles or (in some specimens) parallel-sided and ending at ia. bristles (Figure 4(f))

(Table 3 continued on next page)

(Table 3 continued)

Structure	Species					
	<i>Bactrocera carambolae</i>	<i>Bactrocera caryeae</i>	<i>Bactrocera dorsalis</i> s.l.	<i>Bactrocera kandiensis</i>	<i>Bactrocera occipitalis</i>	<i>Bactrocera pyrifoliae</i>
Wing						
Costal band (Figures 10 and 16)	Narrow, slightly overlapping R ₂₊₃ , moderately broad around apex of wing (Figure 16(a))	Very narrow, confluent with R ₂₊₃ , very narrow around apex of wing (Figure 16(b))	Narrow, generally confluent with R ₂₊₃ (inter- or intra-regionally variable), narrow to moderately broad around apex of wing (Figure 16(c))	Narrow, confluent with R ₂₊₃ , narrow around margin of wing (Figure 16(d))	Narrow, distinctly overlapping R ₂₊₃ , broad around apex of wing extending to mid-point between R ₂₊₃ and R ₄₊₅ (Figure 16(e))	Narrow, confluent with R ₂₊₃ , narrow but slightly expanding around apex of wing (Figure 16(f))
Legs						
Femora (Figure 15)	Fulvous, generally with a large elongate oval black marking on outer surface of fore femora (Figure 15(a))	Fulvous with large dark fuscous markings on all femora (Figure 15(b))	Generally fulvous, occasionally with a small dark marking on outer surface of fore femora (inter-regionally variable) (Figure 15(c))	Fulvous with large dark markings on all femora (Figure 15(d))	Generally fulvous, occasionally with a small preapical dark spot on outer surface of fore femora (Figure 15(e))	Fulvous with a small apical marking on fore femora and dark fuscous around apices of mid and hind femora (Figure 15(f))
Genitalia						
Aculeus length (mm) (Figure 6)	1.3–1.6	n/a	1.4–2.2 (inter- or intra-regionally variable)	n/a	n/a	n/a
Ratio (CuA ₁ /Acul.)	1.4–1.6	n/a	1.0–1.8	n/a	n/a	n/a
Aedeagus length (mm) (Figure 8(d))	2.0–2.7	n/a	2.3–3.5 (inter- or intra-regionally variable)	n/a	n/a	n/a
Ratio (Aed./CuA ₁)	1.2–1.3	n/a	1.2–1.4	n/a	n/a	n/a

Acul., aculeus length; Aed., aedeagus length; CuA₁, first anterior branch of cubitus vein (see Figure 10); ia., intra-alar; n/a, not available; npl., notopleural; R₂₊₃, R₄₊₅, posterior branches of radial vein (see Figure 10).

4.2.4 Diagnostic key to six economically important species belonging to the *Bactrocera dorsalis* complex (adult)

1. Postpronotal lobe yellow with dark anteromedial corner (Figures 19(b) and (d))2
 - Postpronotal lobe entirely yellow (Figures 19(a), (c), (e), (f))3
2. Scutum entirely black (Figure 13(b)), abdominal tergites 3–5 with broad black dorsolateral markings (Figures 17(b) and 18(b)); lateral vittae very narrow (Figure 4(b)) *B. caryeae*
 - Scutum mostly black (Figure 13(d)), abdominal tergites 3–5 with “T” pattern and tergites 4–5 with very narrow anterolateral black markings (Figures 17(d) and 18(d)); lateral vittae narrow (Figure 4(d)) *B. kandiensis*
3. Costal band distinctly overlapping R_{2+3} and expanding broadly around apex of wing reaching mid-point between R_{2+3} and R_{4+5} (Figure 16(e)) *B. occipitalis*
 - Costal band widening slightly (Figure 16(c)) to moderately (Figure 16(a)) around apex of wing4
4. Abdominal tergites 3–5 with broad black dorsolateral markings (Figures 17(f) and 18(f)) *B. pyrifoliae*
 - Abdominal tergites 3–5 without broad black dorsolateral markings5
5. Costal band slightly overlapping R_{2+3} , moderately broad around apex of wing (Figure 16(a)); abdominal tergite 3 with a narrow transverse black band across anterior margin (constituting a “T” pattern), widening to cover lateral margins; tergite 4 with rectangular (occasionally triangular) anterolateral or narrow lateral black markings; tergites 3–5 with medium-width medial longitudinal black stripe (Figures 17(a) and 18(a)) *B. carambolae*
 - Costal band confluent with R_{2+3} , narrow to moderately broad around apex of wing (Figure 16(c)); abdominal tergite 3 exhibits variations from black band across anterior margin (constituting a “T” pattern) to broad lateral bands, tergite 4 without markings or with anterolateral or narrow lateral black margins (occasionally rectangular), tergite 5 without markings or with anterolateral black markings (Figures 17(c) and 18(c)) *B. dorsalis s.l.*

4.3 Molecular identification of *Bactrocera carambolae*

Molecular identification of the six target species has been confounded by their very close genetic relationships and uncertain taxonomy (Boykin *et al.*, 2014; Hendrichs *et al.*, 2015). Molecular tests alone are not recommended for identification of the six species. However, molecular methods can provide useful information to support morphological identifications when new records are reported from the morphological diagnosis. When identifying *B. carambolae* and *B. dorsalis s.l.* specimens using this protocol, a molecular test is necessary for accurate identification whenever adult morphology alone cannot distinguish between the two species.

DNA sequencing of either the internal transcribed spacer 1 (ITS1) or 2 (ITS2) nuclear DNA regions has been proposed as a reliable way to distinguish between the species *B. carambolae* and *B. dorsalis s.l.* (Boykin *et al.*, 2014; Schutze *et al.*, 2015a). The ITS1 method as described by Boykin *et al.* (2014) for distinguishing between the two species is included in the current protocol. This method is designed to diagnose a fly as *B. carambolae* based on the presence of a unique DNA insertion that is not present in *B. dorsalis s.l.* The ITS1 method has not been shown to distinguish *B. carambolae* from all other *Bactrocera dorsalis* complex species. Specificity of the method for *B. carambolae* has been examined using only four species in the *Bactrocera dorsalis* complex: *B. dorsalis s.l.*, *B. occipitalis*, *B. opiliae* and *B. cacuminata*.

In this diagnostic protocol, methods (including references to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. Laboratory

procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4.3.1 DNA extraction for molecular tests

Boykin *et al.* (2014) and Ball and Armstrong (2008) provide protocols for DNA extraction using commercial kits that are useful because small amounts of starting material such as one fruit fly leg can give enough DNA yield and quality for PCR. The methods used to preserve fruit flies for morphological and molecular examination are not the same. Ethanol is a common preservative for fruit fly DNA. Although fruit fly specimens can be preserved in $\geq 95\%$ ethanol at $-20\text{ }^{\circ}\text{C}$ or colder for long-term storage, ethanol can alter the colouring of adult specimens, which can hinder morphological identification. All identifications performed using this protocol require morphological examination. In cases where molecular methods are to be used, it is therefore recommended that a leg be removed and stored in ethanol for DNA extraction and that the remaining specimen be prepared for morphology work. Further examples of methods are provided by Plant Health Australia (2016).

4.3.2 ITS1 PCR and DNA sequencing to distinguish *B. carambolae* from *B. dorsalis s.l.*

The Boykin *et al.* (2014) study compared a large collection of ITS1 sequences from *B. dorsalis s.l.* and *B. carambolae* specimens. Although many primer sets for analysis of ITS1 have been reported in the scientific literature (e.g. Plant Health Australia, 2016), the ITS7/ITS6 primer set reported by Boykin *et al.* (2014) is reported here to simplify comparison with reference sequences from that study and stored in GenBank. Other primer sets that target the same region of ITS1 could also function adequately. None of the published primer sets for this target gene have been tested for reproducibility or sensitivity.

The ITS7 (forward) and ITS6 (reverse) primers are:

ITS7 (5'- GAA TTT CGC ATA CAT TGT AT-3') (Boykin *et al.*, 2014)

ITS6 (5'- AGC CGA GTG ATC CAC CGC T-3') (Armstrong and Cameron, 2000)

PCR can be carried out in 30 μl reactions according to Boykin *et al.* (2014), using the master mix and cycling parameters given in Table 4.

Sanger sequencing of PCR products should be carried out using each primer to generate two independent DNA sequence reads in alternate directions. These sequences should be aligned to identify conflicting information. Chromatograms should be edited to resolve conflicting signals. If multiple peaks at a nucleotide are observed in the sequences generated using both the forward and reverse primers then the site should be assigned as an ambiguous base (i.e. N = A, C, T or G). The final edited sequence should be at least 400 base pairs (bp) in length for data interpretation.

Table 4. Master mix composition, cycling parameters and amplicons for PCR to distinguish *Bactrocera carambolae* from *B. dorsalis* s.l.

Reagents	Final concentration
PCR grade water	–†
PCR buffer	1×
MgCl ₂	2.0 mM
dNTPs	200 µM of each
Primer (forward)	0.2 µM
Primer (reverse)	0.2 µM
DNA polymerase	0.6 U
DNA sample	2 µl
Cycling parameters	
Initial denaturation	94 °C for 2 min
Number of cycles	35
- Denaturation	94 °C for 15 s
- Annealing	60 °C for 20 s
- Elongation	69 °C for 60 s
Final elongation	68 °C for 5 min
Expected amplicons	
Size	500–550 bp (the amplicon size varies for species and individuals)

† For a final reaction volume of 30 µl.

bp, base pairs; PCR, polymerase chain reaction.

4.3.3 Controls for molecular tests

For the test result to be considered reliable, appropriate controls should be considered for each series of nucleic acid extractions and PCR amplifications of the target pest. As a minimum, a positive nucleic acid control and a negative amplification control (no template control) should be used for the ITS1 PCR test.

Positive nucleic acid control. This control is used to monitor the efficiency of the test method (apart from the extraction). Pre-prepared (stored) genomic DNA may be used.

Negative amplification control (no template control). This control is necessary to rule out false positives due to contamination with other genetic material during the preparation of the reaction mixture. PCR grade water that was used to prepare the reaction mixture is added in place of template DNA.

Negative extraction control. This control is used to monitor contamination during nucleic acid extraction. This requires processing extraction blanks alongside the samples to be tested.

4.3.4 Interpretation of molecular test results

The size of ITS1 is different for *B. carambolae* and *B. dorsalis* because of a 44-bp insertion in *B. carambolae* located near the binding site of the ITS7 primer. The inserted DNA is identical in all *B. carambolae* studied. The sequence of the insertion is:

5'- GAA AAA TTA ATA AAA AGT TAA ATG ATC TTT TTA TAA AAA AT-3'

The ITS1 sequence is variable between conspecific specimens of these two species (Boykin *et al.*, 2014). Consequently, an identical match for sites outside of the insertion region is not expected.

However, the test sequence should be at least 99% similar to one of the reference sequences for the interpretation to proceed. It is possible to distinguish between *B. carambolae* and *B. dorsalis s.l.* after comparing the DNA sequence of the tested specimen with a representative sequence of each species: GenBank KC446737 for *B. carambolae* and KC446776 for *B. dorsalis s.l.* If the tested sequence is most similar to *B. carambolae* and has the 44-bp insertion region, then it can be diagnosed as *B. carambolae*. If the tested sequence is most similar to *B. dorsalis s.l.* and lacks the insertion region, then it is diagnosed as not *B. carambolae*. Several other species in the *B. dorsalis* complex lack the insertion and a match with *B. dorsalis s.l.* cannot exclude those as a possible identification.

4.4 Other molecular methods of identification

Plant Health Australia (2016) has compiled a resource for identification of *Bactrocera* species using DNA methods. That resource summarizes three molecular options for identification: conventional PCR and restriction fragment length polymorphism (RFLP) of the ITS1 region (Plant Health Australia, 2016), PCR-RFLP analysis of a segment of ribosomal DNA array including the ITS1 and 18S gene regions (Armstrong *et al.*, 1997; Armstrong and Cameron, 2000), and DNA barcoding of the *cytochrome oxidase subunit I (COI)* gene (Armstrong and Ball, 2005) based on the Barcode of Life Data Systems resource (Ratnasingham and Hebert, 2007). Molecular profiles for the species *B. caryae*, *B. kandiensis*, *B. occipitalis* and *B. pyrifoliae* are not available for either of the PCR-RFLP methods described in the Plant Health Australia resource, precluding the use of these methods as a diagnostic test for these pests.

DNA barcode records of the *COI* gene are not available for *B. pyrifoliae*, and cannot distinguish the other five species from each other (Armstrong and Ball, 2005). The work by Leblanc *et al.* (2015) demonstrates that this complex is not a monophyletic group and a molecular identification of the complex is not possible using *COI* sequence data.

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be adversely affected by the diagnosis, records and evidence (in particular, preserved or slide-mounted specimens, photographs of distinctive taxonomic structures, DNA extracts and photographs of gels, as appropriate) should be kept for at least one year in a manner that ensures traceability.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Pest Identification and Diagnostics Section, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan (Kenji Tsuruta; email: tsurutak@pps.maff.go.jp; tel.: +81-45-622-8940; fax: +81-45-621-7560).

Regional R&D Training Center for Insect Biotechnology (RCIB), Department of Biotechnology, Mahidol University, 272 Rama VI Road, Ratchathewee, Bangkok 10400, Thailand (Sujinda Thanaphum; email: sujinda.tha@mahidol.ac.th; tel.: +66814333963; fax: +6623547160).

William F. Barr Entomological Museum, Department of Plant, Soil and Entomological Sciences, University of Idaho, 875 Perimeter Drive MS 2339, Moscow, ID 83844-2339, United States of America (Luc Leblanc; email: leblanc1@uidaho.edu; tel.: +1 208-885-6274; fax: +1 208-885-7760).

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

The first draft of this protocol was written by Kenji Tsuruta (Ministry of Agriculture, Forestry and Fisheries, Japan (see preceding section)), Sujinda Thanaphum (Mahidol University, Thailand (see preceding section)), Luc Leblanc (University of Idaho, United States of America (see preceding section)) and Norman Barr (United States Department of Agriculture, United States of America). The following experts provided comments on earlier versions that improved the quality of the protocol: Jane Royer (Queensland Department of Agriculture and Fisheries, Australia), Mark Schutze (Queensland University of Technology, Australia), Josephine Moraa Songa (Kenya Agricultural & Livestock Research Organization, Kenya), George Momanyi (Kenya Plant Health Inspectorate Service, Kenya), Sharon Reid (Fera Science Ltd., Sand Hutton, York, United Kingdom), Yuji Kitahara (Ministry of Agriculture, Forestry and Fisheries, Japan), Eddy Dijkstra (Plant Protection Service, Netherlands) and Ken Hong Tan (Tan Hak Heng, Penang, Malaysia).

8. References

The present annex may refer to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at <https://www.ippc.int/core-activities/standards-setting/ispms>.

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9. Figures



Figure 1. *Bactrocera dorsalis s.l.*, female (habitus).

Source: Photo courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

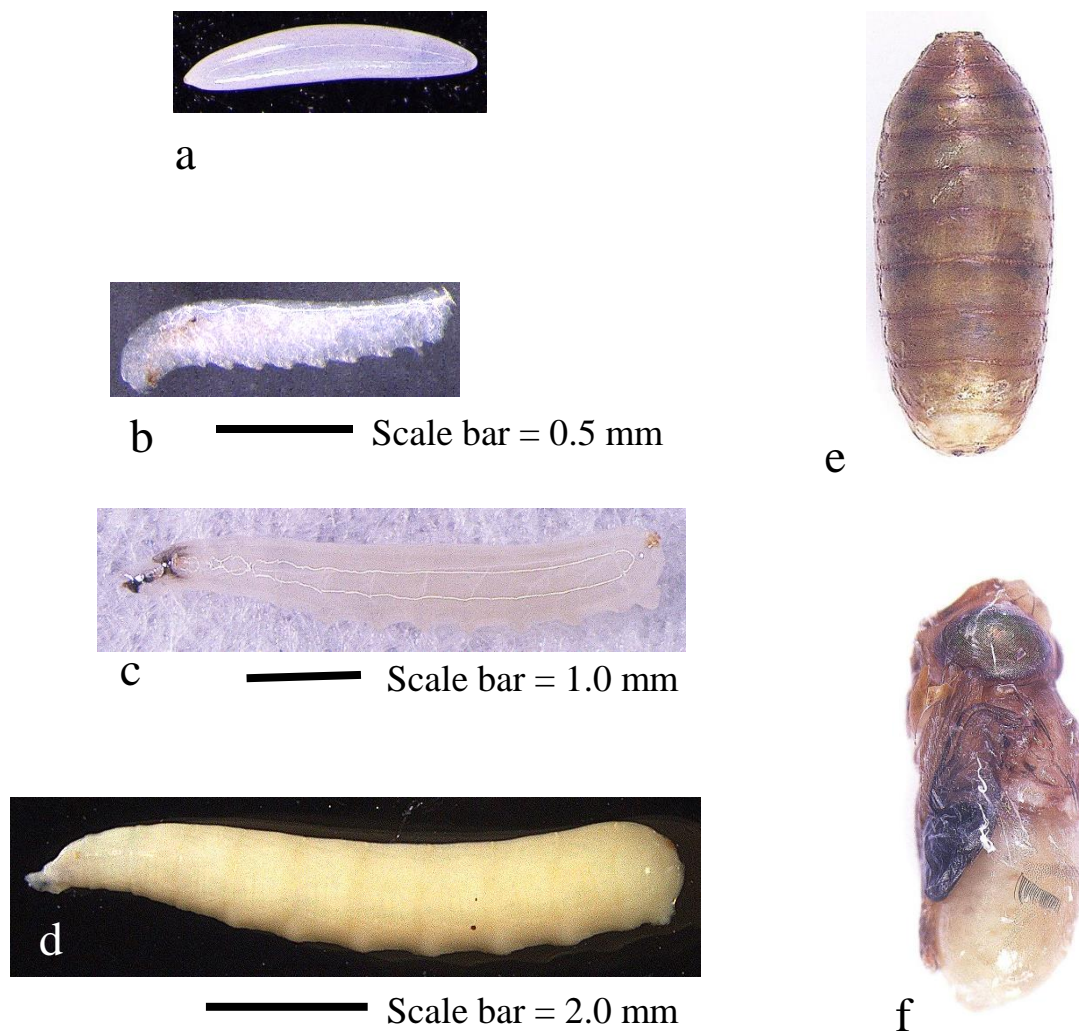


Figure 2. Immature stages of *Bactrocera dorsalis* s.l.: (a) egg; (b) first instar larva; (c) second instar larva; (d) third instar larva; (e) puparium; (f) pupa.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

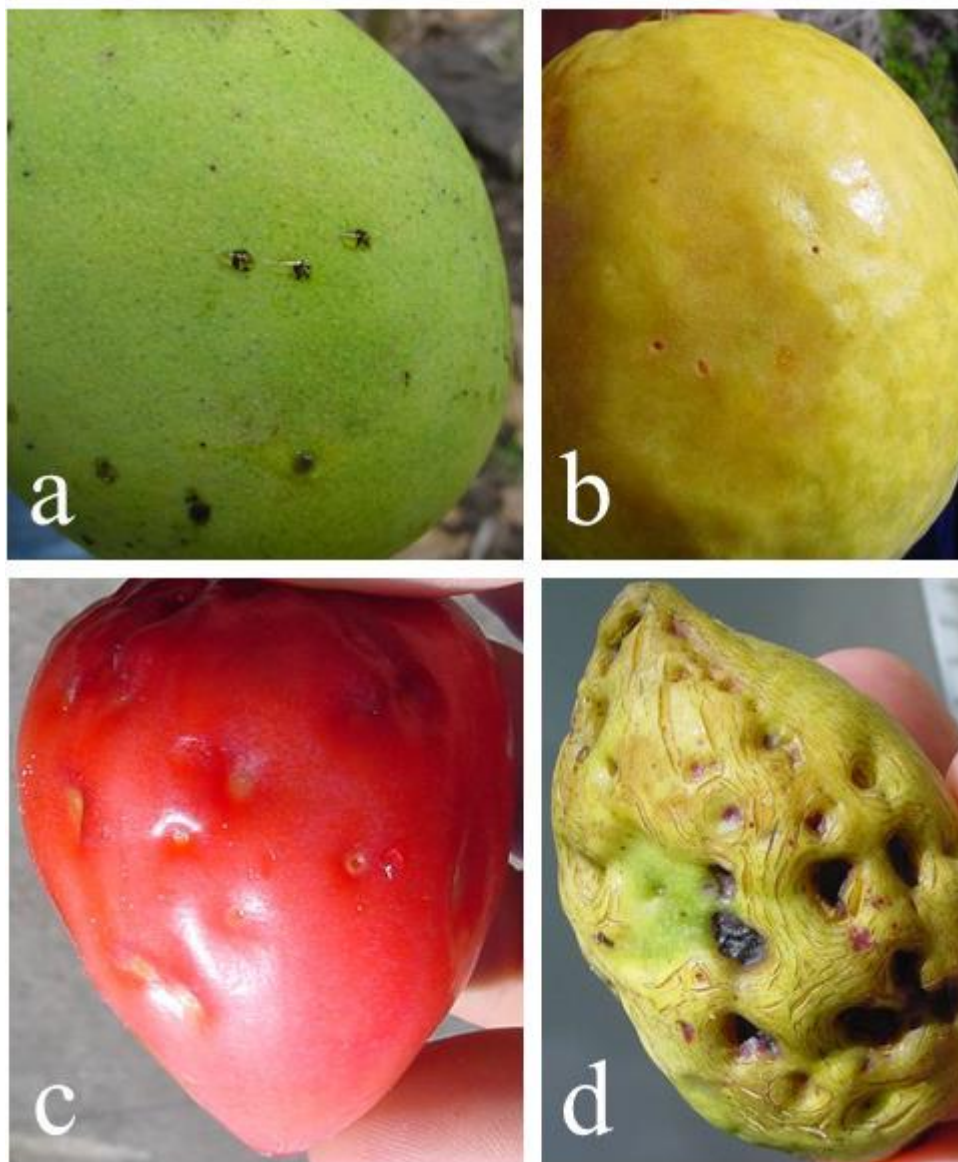


Figure 3. Oviposition punctures on (a) *Mangifera* spp. (mango), (b) *Psidium* spp. (guava), (c) *Syzygium samarangense* (java apple), (d) *Terminalia catappa* (tropical almond).
Source: Photos courtesy of Luc Leblanc, University of Idaho, United States of America.

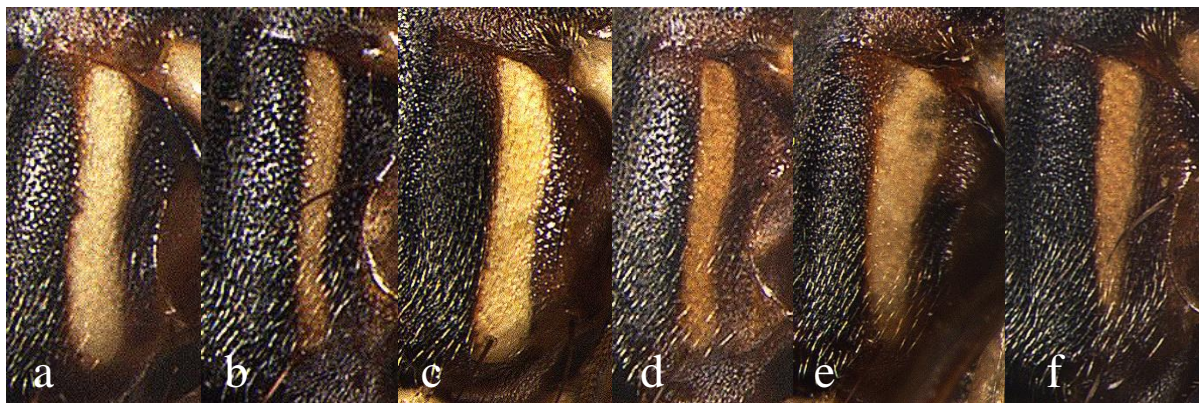


Figure 4. Lateral vitta: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
 Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

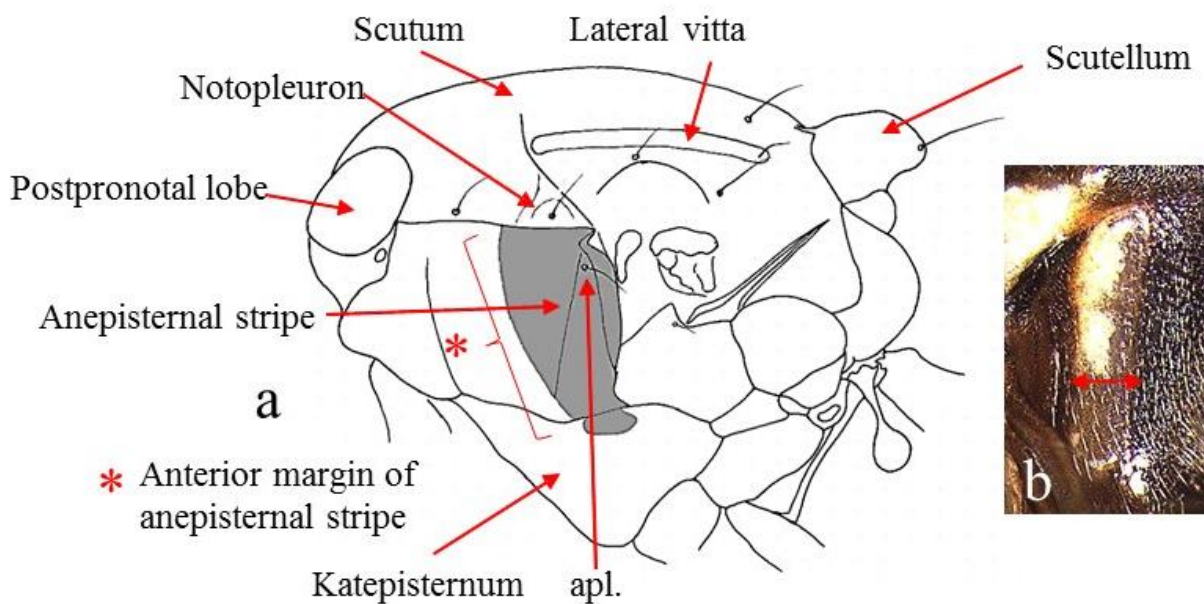


Figure 5. (a) Lateral view of Dacinae thorax. (b) Damaged lateral vitta, showing translucent window.
 apl., anepisternal bristle.
 Source: Photo and line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

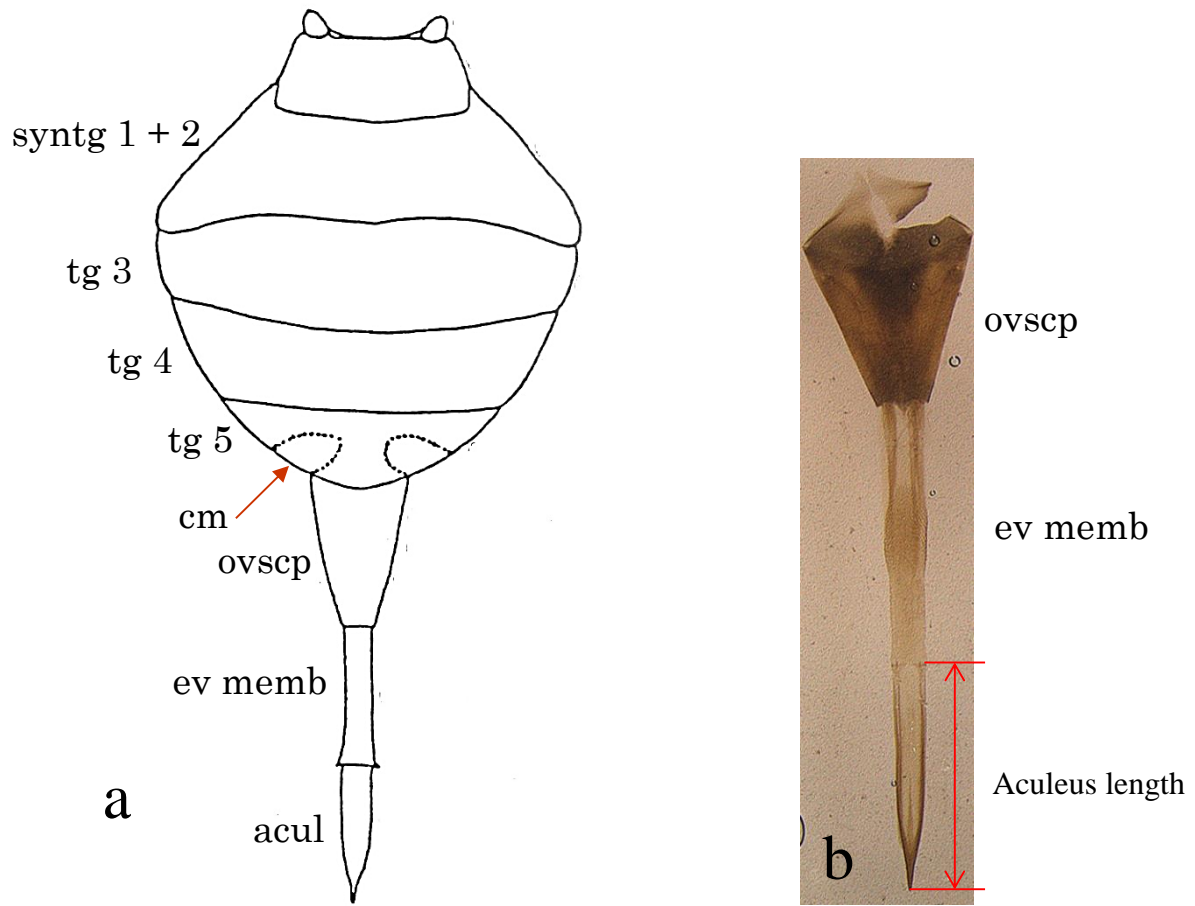


Figure 6. Dacinae abdomen: (a) female in dorsal view; (b) genitalia (fully extended). acul, aculeus; cm, ceromata; ev memb, eversible membrane; ovscp, oviscape; syntg 1 + 2, syntergites 1 + 2; tg3, tergite 3; tg4, tergite 4; tg5, tergite 5.

Source: Line drawing (a) adapted from Ito (1988) and photo (b) courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

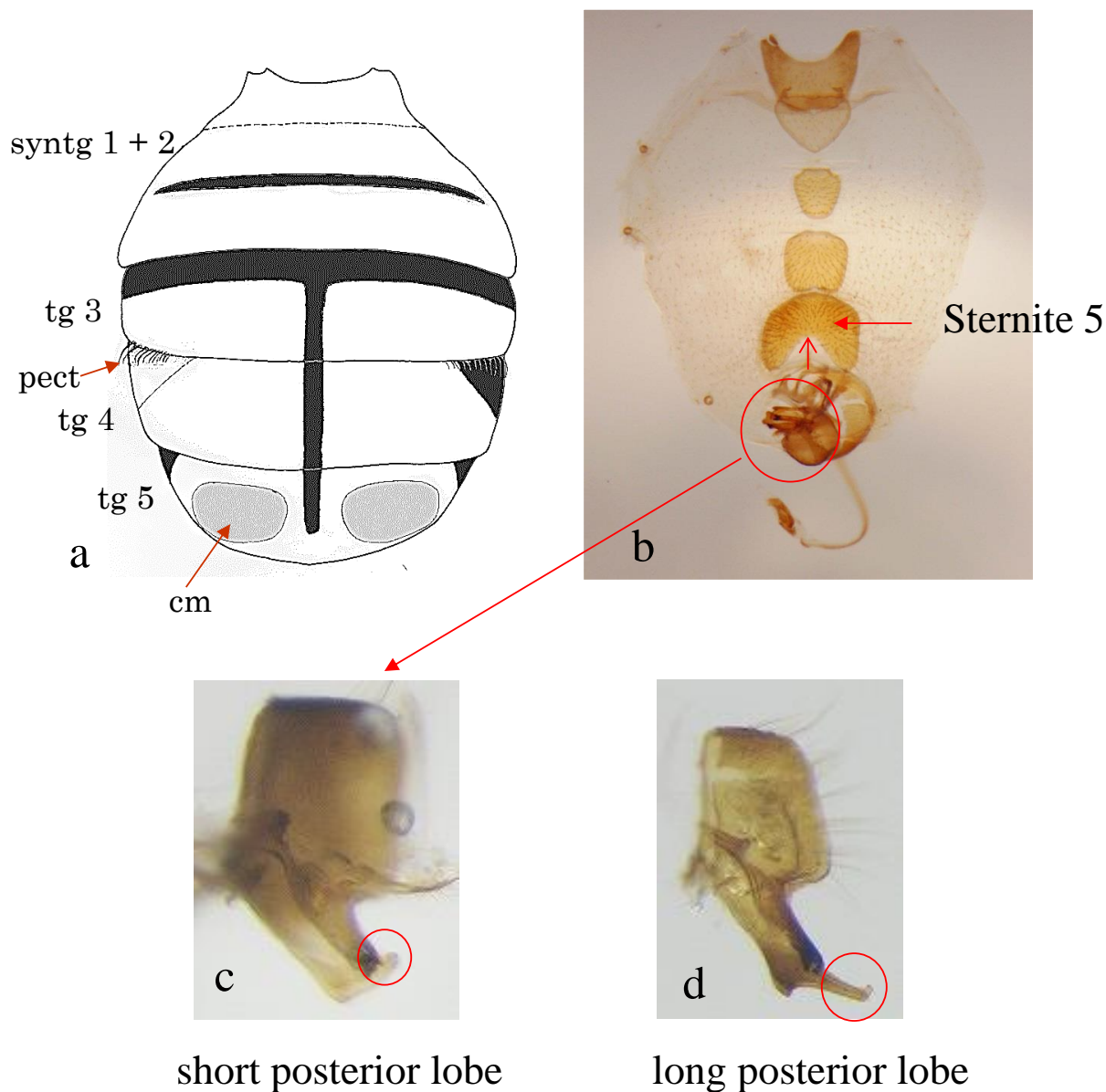


Figure 7. Dacinae abdomen: (a) male in dorsal view; (b) male in ventral view; (c) epandrium and lateral surstylus, showing short posterior lobe; (d) epandrium and lateral surstylus, showing long posterior lobe. cm, ceromata; pect, pecten; syntg 1 + 2, syntergites 1 + 2; tg3, tergite 3; tg4, tergite 4; tg5, tergite 5.

Source: Photos and line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

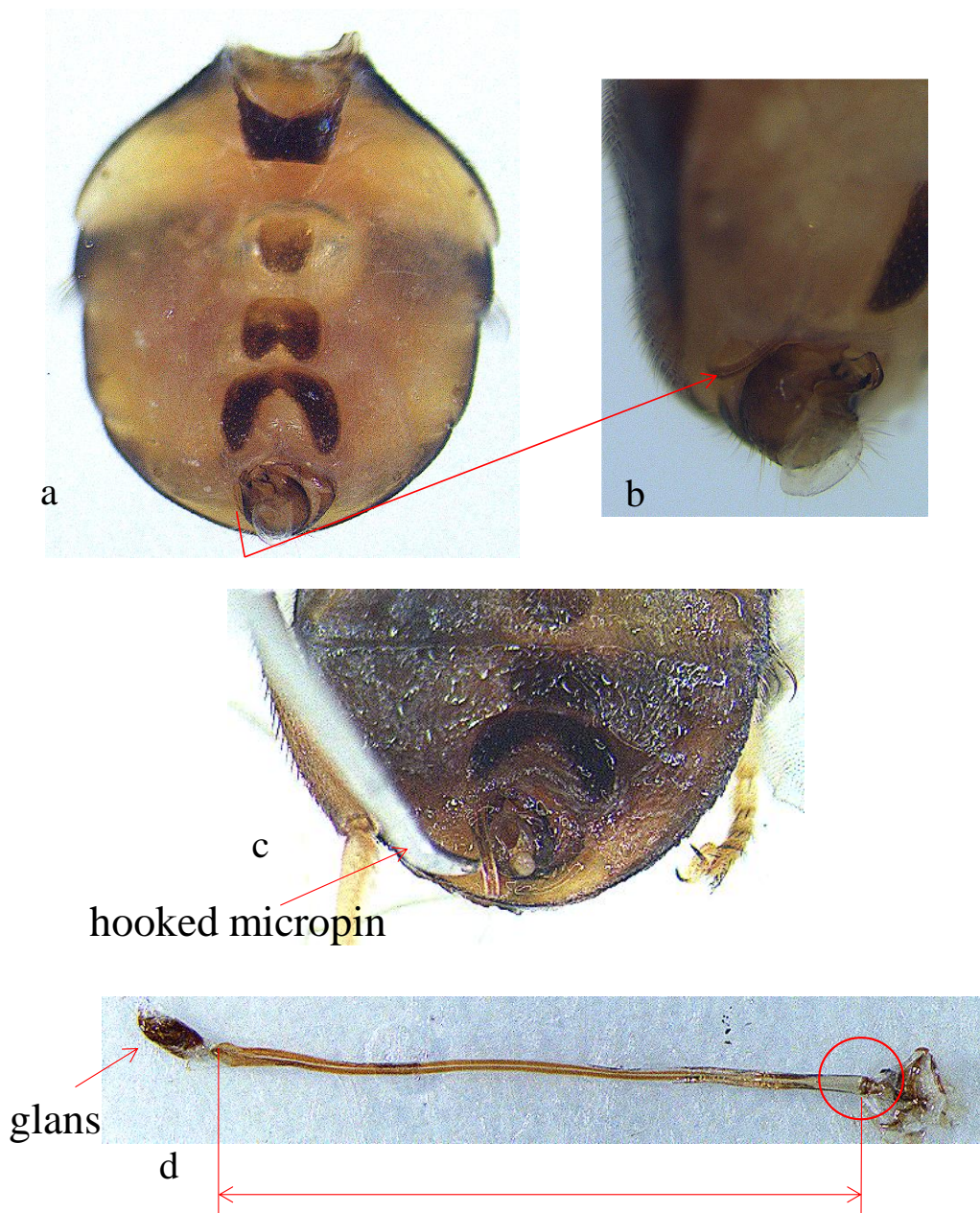


Figure 8. Male abdomen and aedeagus (*B. dorsalis s.l.*): (a) abdomen in ventral view (KOH treated); (b) part of aedeagus appearing rightside (when base of abdomen set upside-down and viewed from ventral side) of epandrium; (c) pulling out aedeagus using hooked micropin; (d) extended aedeagus, showing the part to be measured.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

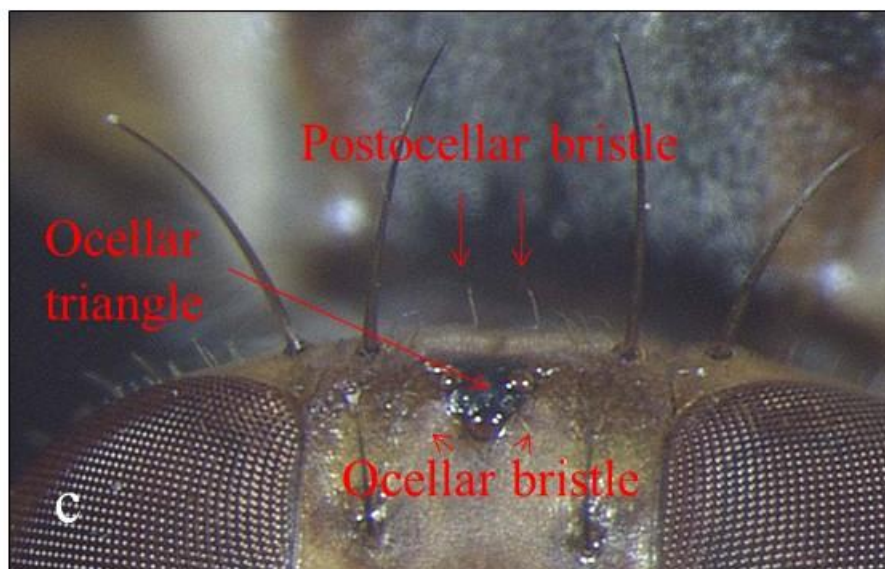
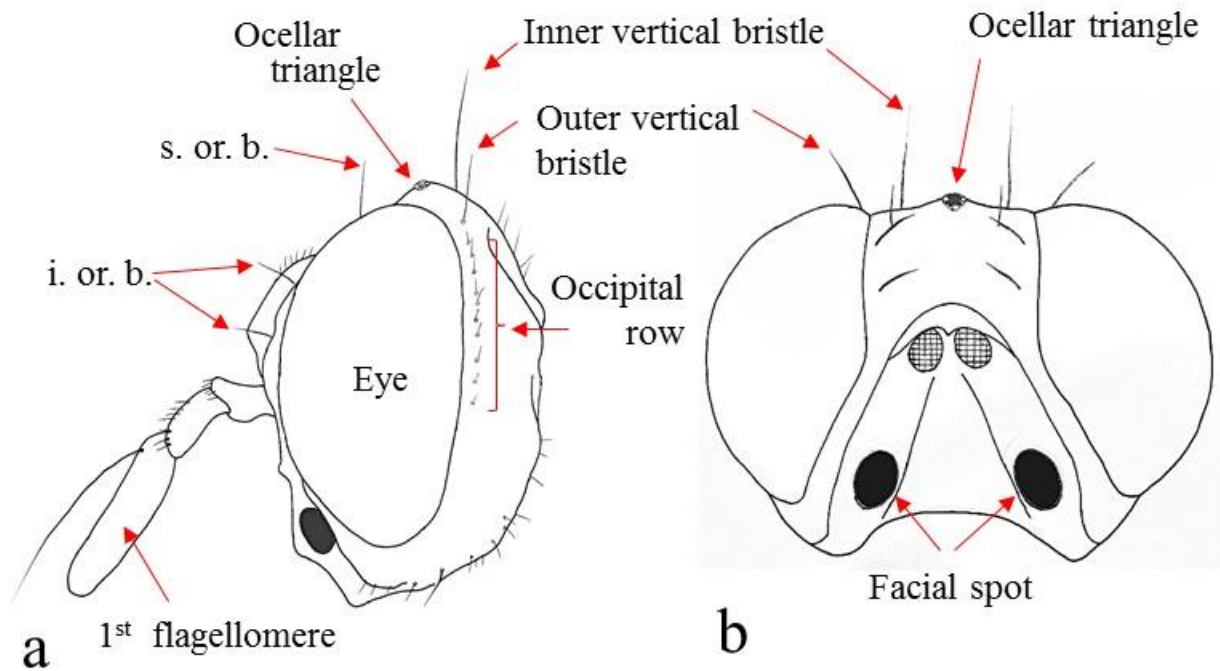


Figure 9. (a) Lateral view of Dacinae head. (b) Frontal view of Dacinae head. (c) Dorsal view of Dacinae head (vertex). i. or. b., inferior fronto-orbital bristles; s. or. b., superior fronto-orbital bristles.
 Source: Photo and line drawings courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

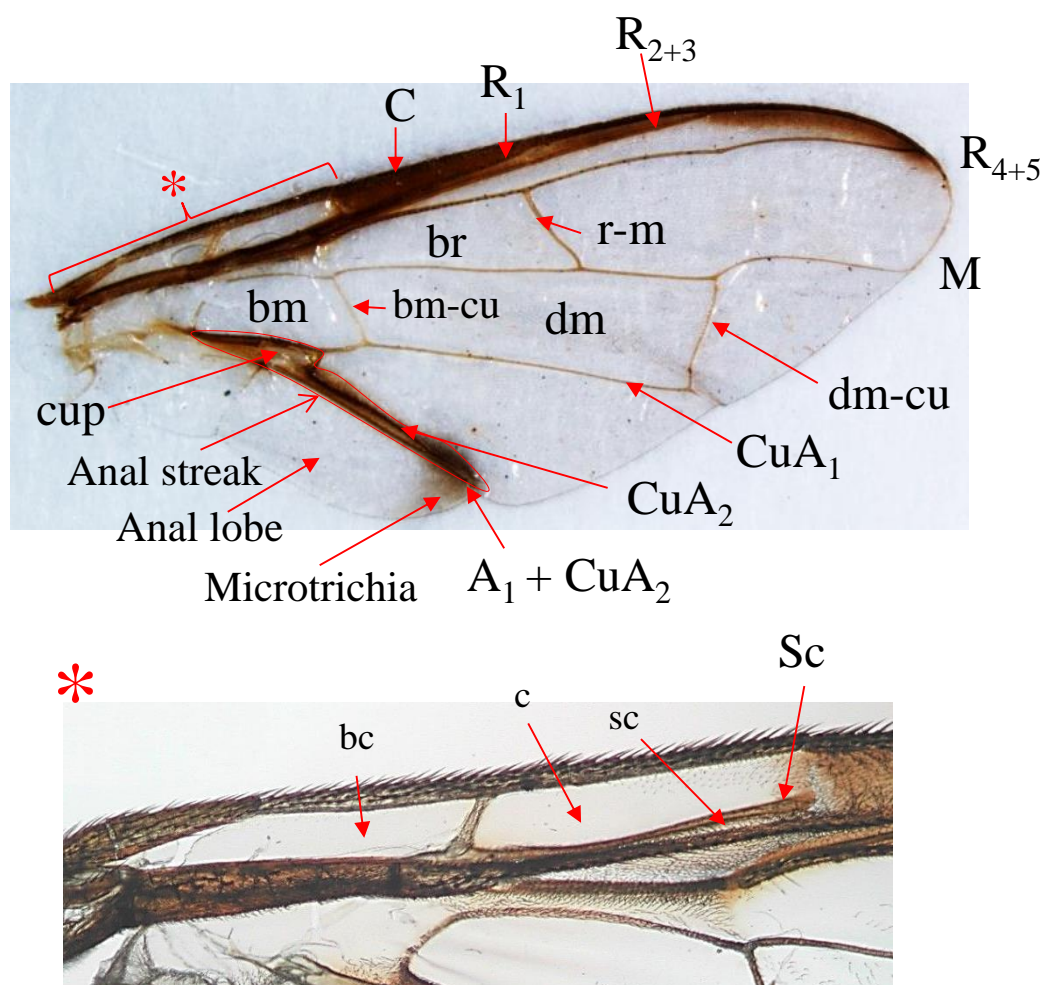


Figure 10. Wing of Dacinae (top) with a magnified view of c and bc cells marked by asterisk (bottom). Veins: A₁, branch of anal vein; bm-cu = basal medial-cubital crossvein; C, costa; CuA₁, CuA₂, anterior branches of cubitus; dm-cu, discal medial-cubital crossvein; M, media; R₁, anterior branch of radius; R₂₊₃, R₄₊₅, combined posterior branches of radius; r-m, radial-medial crossvein; Sc, subcosta. Cells: bc, basal costal; bm, basal medial; br, basal radial; c, costal; cup, posterior cubital; dm, discal medial; sc, subcostal. Anal streak, areas around cup and cup extension indicated by red outline.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

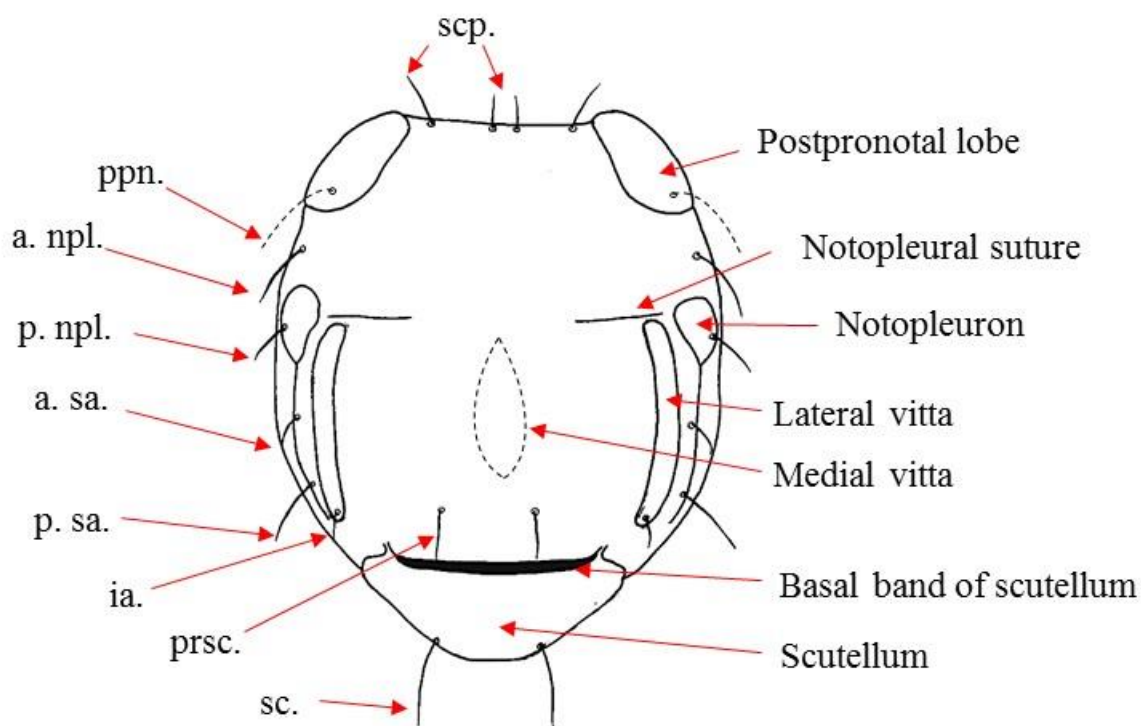


Figure 11. Dorsal view of Dacinae thorax. a. npl., anterior notopleural bristle; a. sa., anterior supra-alar bristle; ia., intra-alar bristle; p. npl., posterior notopleural bristle; ppn., postpronotal bristle; prsc., prescutellar bristle; p. sa., posterior supra-alar bristle; sc., scutellar bristle; scp., scapular bristle.

Source: Line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

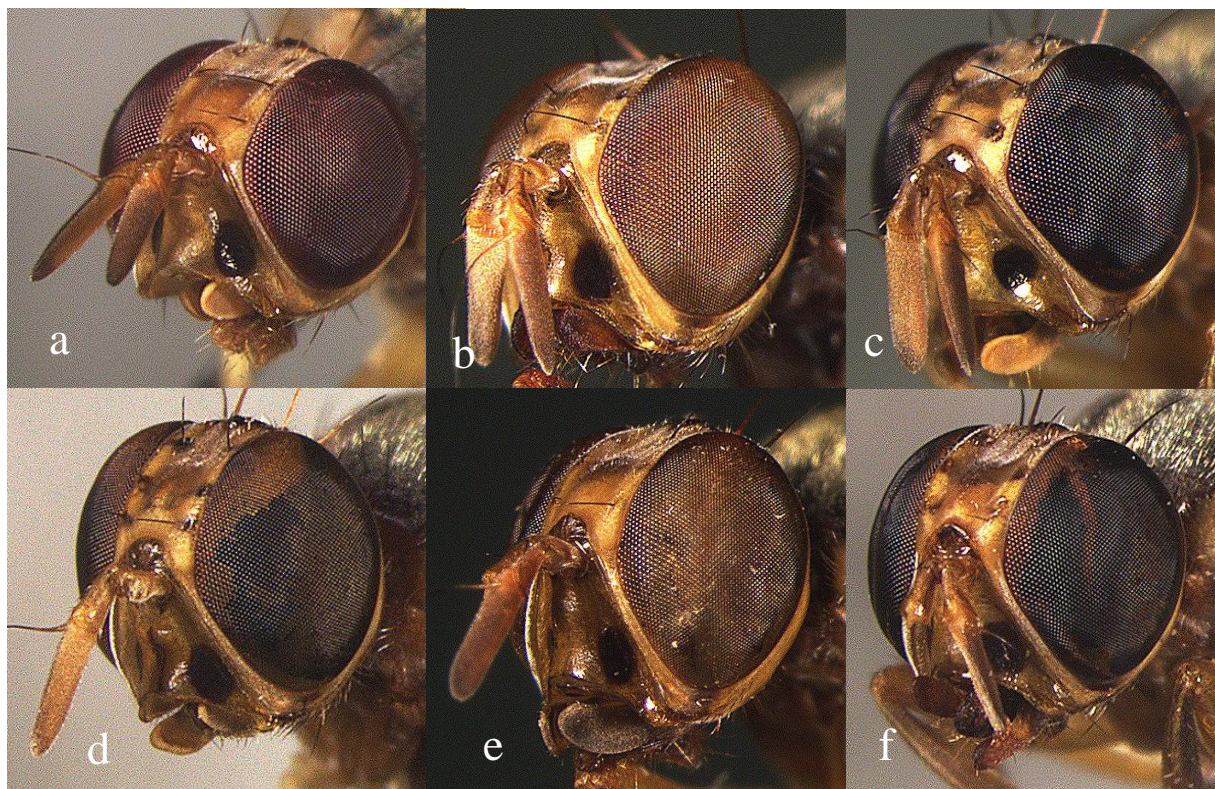


Figure 12. Head in anterolateral view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

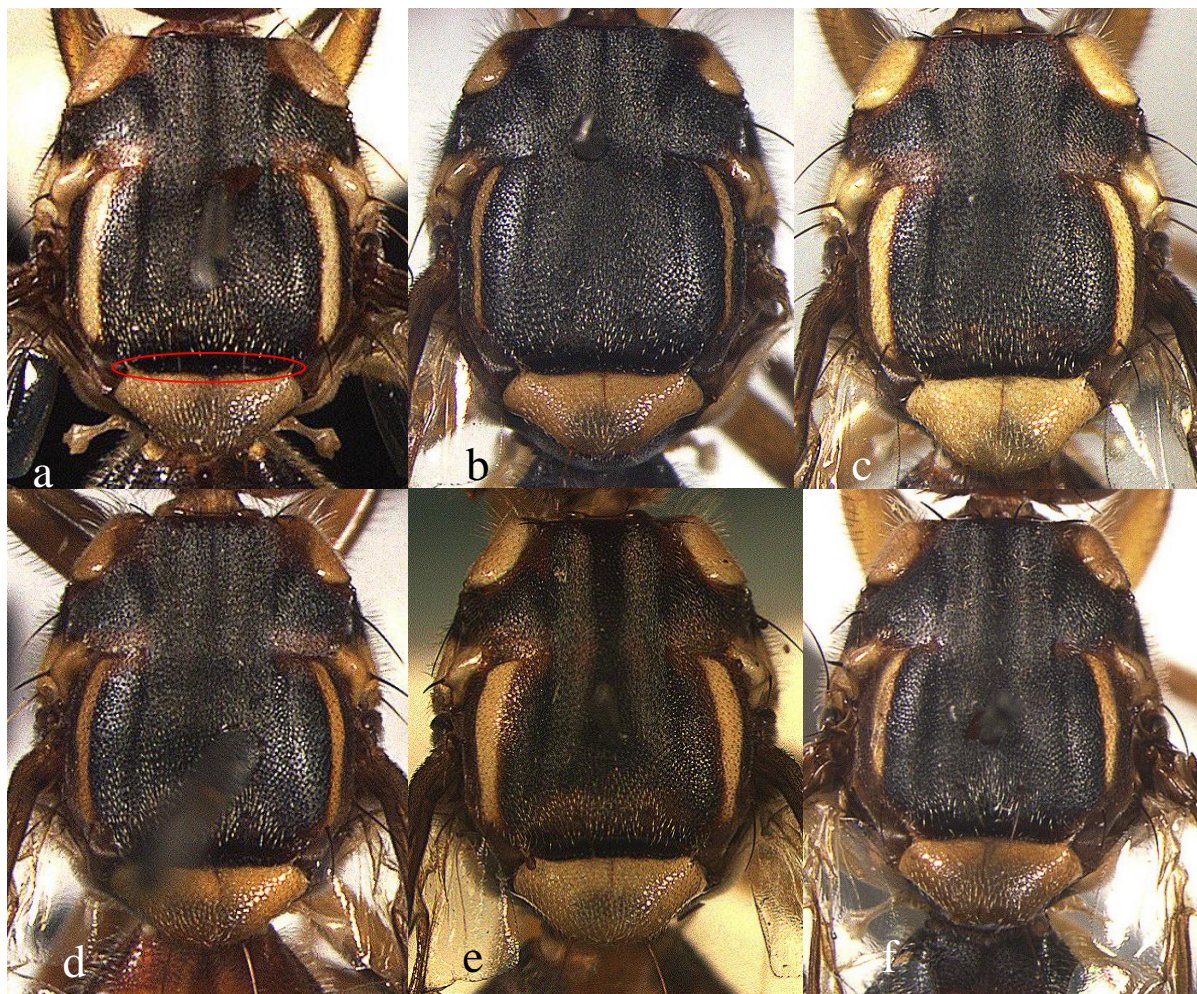
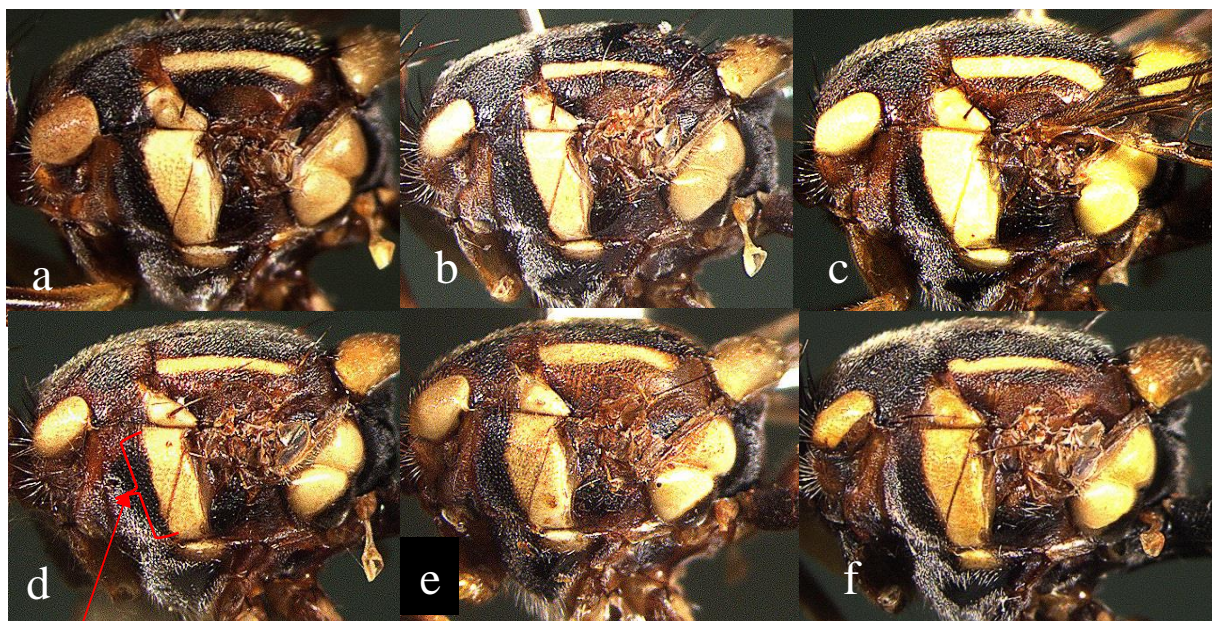


Figure 13. Thorax in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*. Basal band indicated by red circle in image (a).

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.



Anterior margin of anepisternal stripe

Figure 14. Thorax in lateral view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*. The margin of the episternal stripe is marked in (d).

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.



Figure 15. Legs: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*. 1, fore leg (outer surface); 2, mid leg; 3, hind leg (inner surface, when folded back alongside abdomen).

Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

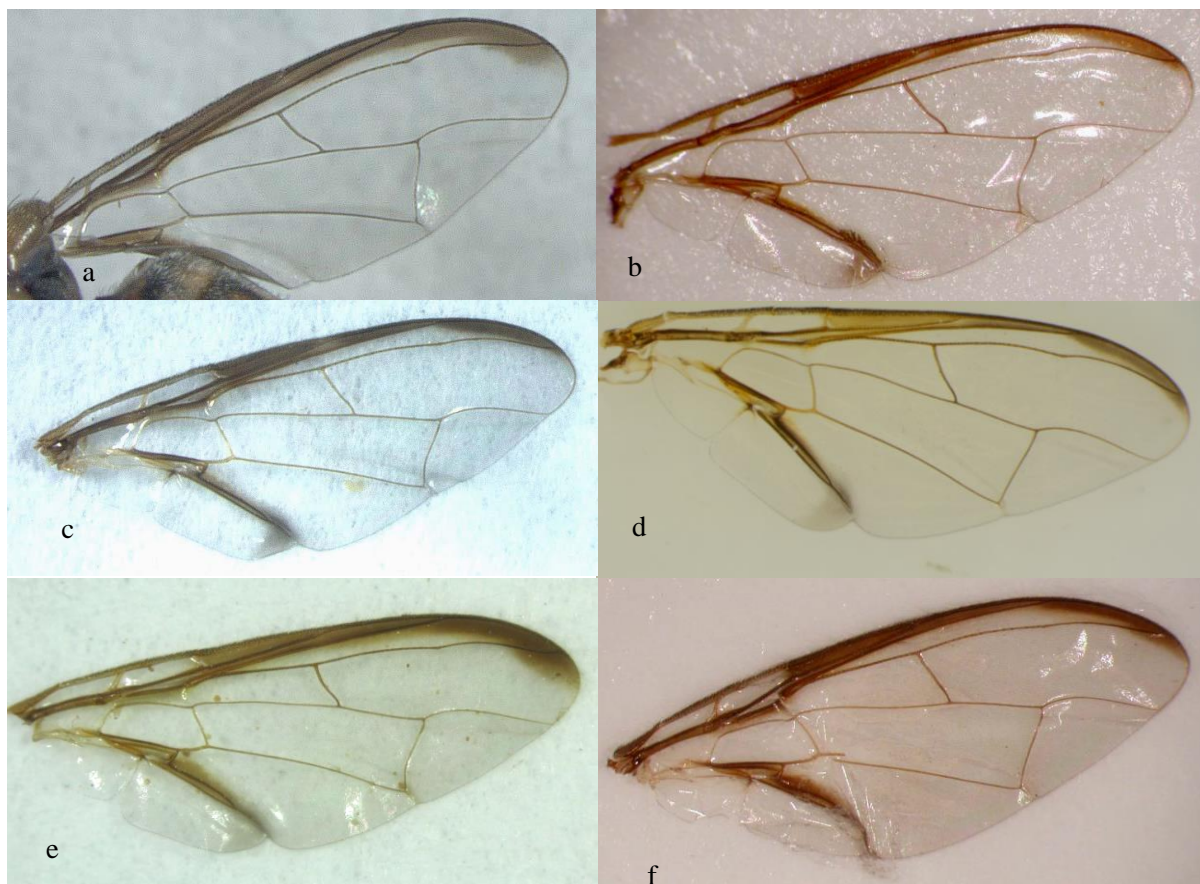


Figure 16. Wings: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.

Source: Photos (a–c, e–f) courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan; (d) courtesy of Luc Leblanc, University of Idaho, United States of America.

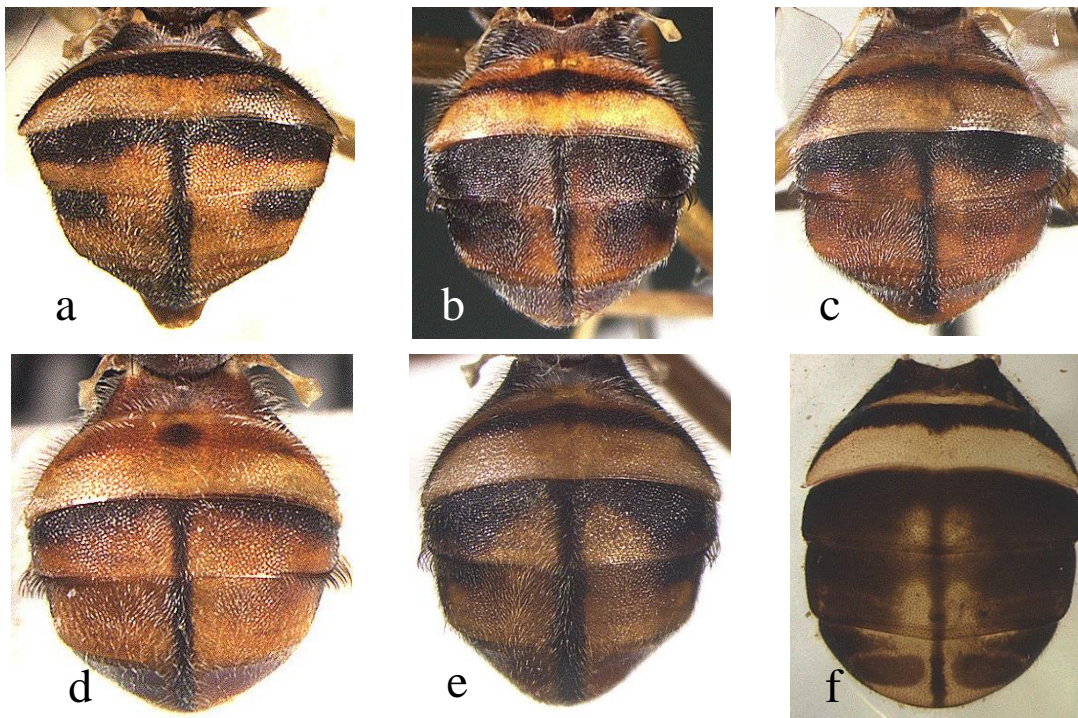


Figure 17. Abdomen in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyriformae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

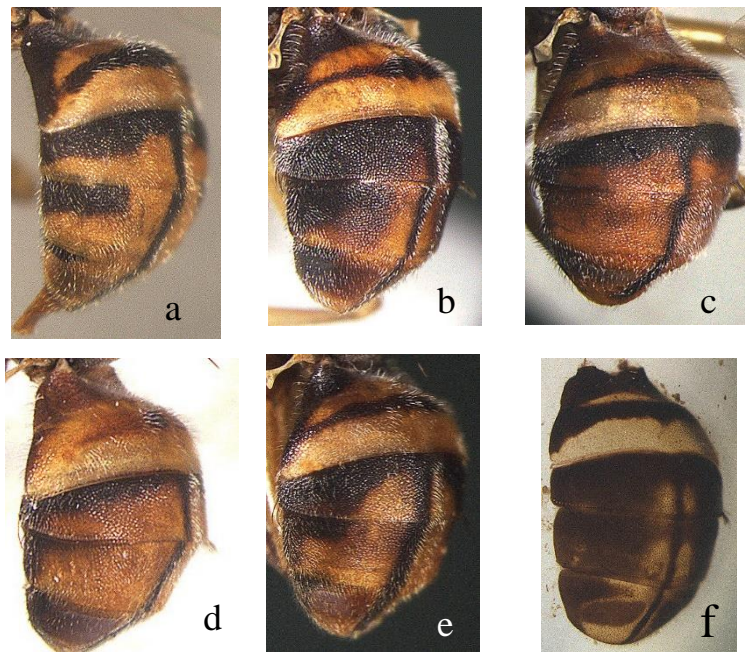


Figure 18. Abdomen in dorsolateral view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis* s.l.; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyriformae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

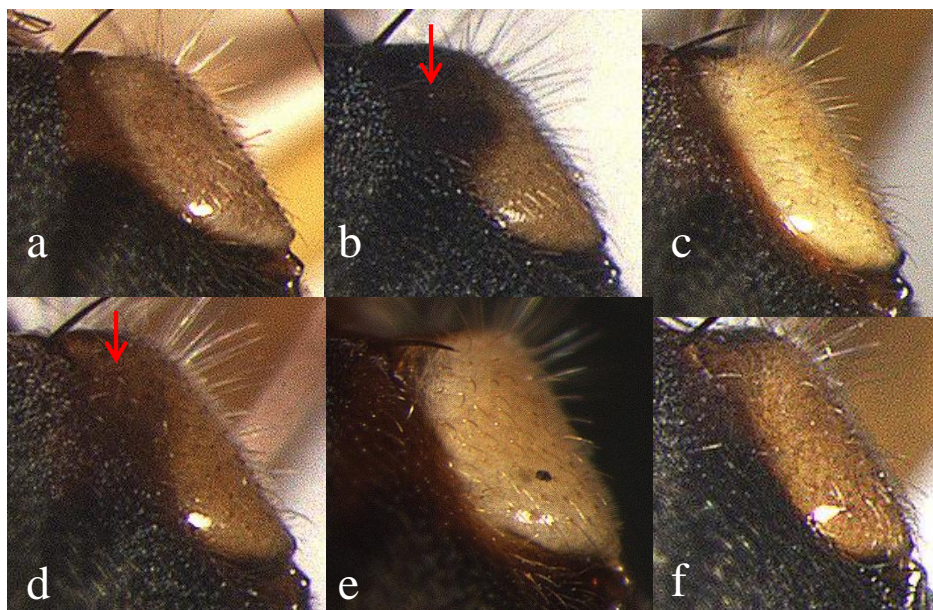


Figure 19. Postpronotal lobes in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

Publication history

This is not an official part of the standard

2006-05 Standards Committee (SC) added original subject: *Bactrocera dorsalis* complex (2006-026).

2016-10 Expert consultation.

2017-02 Technical Panel on Diagnostic Protocols (TPDP) review.

2017-05 TPDP approved to submit to SC for consultation (2017_eTPDP_05).

2017-06 SC approved draft to be submitted for consultation (2017-eSC_Nov_01).

2017-07 First consultation.

2017-11 Reviewed by the Lead based on consultation comments.

2018-02 TPDP approved to submit to SC for approval for adoption.

2018-03 SC approved draft to be submitted to the 45-day DP notification period (2018_eSC_May_06).

2018-07 DP notification period. Objection received.

2018-09 DP drafting group revised.

2018-10 TPDP approved to submit to SC for approval for adoption.

2018-11 SC approved draft to be submitted to the 45-day DP notification period.

2019-01 DP notification period (no objections received).

2019-02 SC adopted DP on behalf of CPM.

ISPM 27. Annex 29. *Bactrocera dorsalis* (2019). Rome, IPPC, FAO.

Publication history last updated: 2019-03